WHAT IS CLAIMED IS:

1   1.   An *in vivo* method of affinity maturation by competitive activation to

2 obtain a binding molecule that has an enhanced affinity for a target binding ensemble

3 member relative to that of a reference binding molecule, the method comprising:

4   (a) recombinantly altering a population of host cells by

5   (i) introducing into the host cells a library of genes encoding candidate

6 binding molecules;

7   (ii) introducing into the host cells a competitive activation system

8 comprising a nucleic acid encoding a responder molecule linked to the target binding

9 ensemble member, and a nucleic acid encoding a competitor binding molecule linked to an

10 inhibitor of the responder complex;

11   (b) incubating the host cells under conditions in which the library and

12 competitive activation system are expressed and where the responder molecule is activated

13 when a candidate binding molecule binds to the target binding ensemble member; and

14   (c) detecting cells having a signal from the responder molecule that

15 corresponds to a candidate binding molecule binding affinity for the target binding ensemble

16 member that is greater than that of the reference binding molecule, thereby identifying a

17 candidate binding molecule with an enhanced affinity for the target binding ensemble

18 member.

1   2.   The method of claim 1, wherein the reference binding molecule is a

2 reference antibody and the target binding ensemble member is an antigen to which the

3 reference antibody specifically binds.

1   3.   The method of claim 2, further wherein the competitor binding

2 molecule is the reference antibody.

1   4.   The method of claim 3, wherein the reference antibody is an Fab

2 fragment.

1   5.   The method of claim 3, wherein the reference antibody is a single

2 chain Fv.

1   6.   The method of claim 2, further wherein the candidate binding

2 molecules are single chain Fvs.

1   7.   The method of claim 2, further wherein the candidate binding
2 molecules are Fab fragments.

1   8.   The method of claim 2, further wherein the candidate binding
2 molecules are single V-region domains.

1   9.   The method of claim 1, wherein the candidate binding molecules are
2 scaffolded peptides.

1   10.   The method of claim 1, wherein the candidate binding molecules are
2 mutagenized natural ligands of the target binding ensemble member.

1   11.   The method of claim 2, further wherein the library of candidate
2 binding molecules comprises hybrid antibodies that have at least one CDR in a V_H or V_L that
3 is different from the reference antibody and is from a natural antibody repertoire.

1   12.   The method of claim 11, wherein the hybrid antibodies have either a
2 V_H or V_L from the reference antibody and the corresponding V_H or V_L from a natural
3 antibody repertoire.

1   13.   The method of claim 2, further wherein the competitor binding
2 molecule is a nonhuman antibody and the candidate binding molecules are antibodies having
3 at least one human variable region.

1   14.   The method of claim 2, further wherein the competitor binding
2 molecule is a natural ligand of the antigen that competes with the reference antibody for
3 binding to the antigen.

1   15.   The method of claim 2, wherein the competitor binding molecule is an
2 artificial non-antibody ligand of the antigen that competes with the reference antibody for
3 binding to the antigen.

1   16.   The method of claim 1, wherein the responder molecule is an enzyme.

1   17.   An *in vivo* method of affinity maturation by competitive activation to
2 obtain a binding molecule that has an enhanced affinity for a target binding ensemble
3 member relative to that of a reference binding molecule, the method comprising:

4   (a) recombinantly altering a population of host cells by

5   (i) introducing into the host cells a library of genes encoding candidate

6 binding molecules;

7   (ii) introducing into the host cells a competitive activation system

8 comprising a nucleic acid encoding a responder molecule linked to a competitor binding

9 molecule, and a nucleic acid encoding an inhibitor linked to the target binding ensemble

10 member;

11   (b) incubating the host cells under conditions in which the library and

12 competitive activation system are expressed and where the responder molecule is activated

13 when a candidate binding molecule binds to the target binding ensemble member; and

14   (c) detecting cells having a signal from the responder molecule that

15 corresponds to a candidate binding molecule affinity for the target ensemble member that is

16 greater than that of the reference binding molecule, thereby identifying a candidate binding

17 molecule with an enhanced affinity for the target binding ensemble member.

1   18.   The method of claim 17, wherein the reference binding molecule is a

2 reference antibody and the target binding ensemble member is an antigen to which the

3 reference antibody specifically binds.

1   19.   The method of claim 18, further wherein the competitor binding

2 molecule is the reference antibody.

1   20.   The method of claim 19, wherein the reference antibody is an Fab

2 fragment.

1   21.   The method of claim 19, wherein the reference antibody is a single

2 chain Fv.

1   22.   The method of claim 18, further wherein the candidate binding

2 molecules are single chain Fvs.

1   23.   The method of claim 18, further wherein the candidate binding

2 molecules are Fab fragments.

1   24.   The method of claim 18, further wherein the candidate binding

2 molecules are single V-region domains.

1   25.   The method of claim 17, further wherein the candidate binding

2 molecules are scaffolded peptides.

1   26.   The method of claim 17, further wherein the candidate binding

2 molecules are mutagenized natural ligands of the target binding ensemble member.

1   27.   The method of claim 18, further wherein the library of candidate

2 binding molecules comprises hybrid antibodies that have at least one CDR in a V_H or V_L that

3 is different from the reference antibody and is from a natural antibody repertoire.

1   28.   The method of claim 27, wherein the hybrid antibodies have either a

2 V_H or V_L from the reference antibody and the corresponding V_H or V_L from a natural

3 antibody repertoire.

1   29.   The method of claim 18, further wherein the competitor binding

2 molecule is a nonhuman antibody and the candidate binding molecules are antibodies having

3 at least one human variable region.

1   30.   The method of claim 18, further wherein the competitor binding

2 molecule is an artificial non antibody ligand of the antigen that competes with the reference

3 antibody for binding to the antigen.

1   31.   The method of claim 18, wherein the competitor binding molecule is

2 an artificial non-antibody ligand of the target antigen that competes with the reference

3 antibody for binding to the target antigen.

1   32.   The method of claim 18, wherein the responder molecule is an

2 enzyme.

1   33.   An *in vivo* method of affinity maturation by auto-inhibited reactivation

2 to obtain a binding molecule that has an enhanced affinity for a target binding ensemble

3 member relative to a reference binding molecule, the method comprising:

4   (a) recombinantly altering a population of host cells by

5   (i) introducing into the host cells a competitor that binds to the target

6 binding ensemble member with the same specificity as a reference binding molecule;

7   (ii) introducing into the host cells a nucleic acid encoding a reactivator

8   complex comprising a reactivator molecule linked to the target binding ensemble member;

9   (iii) introducing into the host cells a library of genes, each of which

10   encodes an auto-inhibited responder complex comprising a responder molecule linked to an

11   inhibitor and linked to a candidate binding molecule;

12   (b) incubating the host cells under conditions in which the competitor, the

13   reactivator complex, and the auto-inhibited responder library are expressed where the

14   responder molecule is activated when a candidate binding molecule binds to the target

15   binding ensemble member; and

16   (c) detecting cells having a signal from the responder molecule that

17   corresponds to a candidate binding molecule affinity for the target binding ensemble member

18   that is greater than that of the reference binding molecule, thereby identifying a candidate

19   binding molecule with an enhanced affinity for the target binding ensemble member.

1   34.   The method of claim 33, wherein the reference binding molecule is an

2   antibody and the target binding ensemble member is an antigen to which the reference

3   antibody specifically binds.

1   35.   The method of claim 34, further wherein the competitor is the

2   reference antibody.

1   36.   The method of claim 35, further wherein the reference antibody is an

2   Fab fragment.

1   37.   The method of claim 35, further wherein the reference antibody is a

2   single chain Fv (scFv).

1   38.   The method of claim 34, further wherein the candidate binding

2   molecules are single chain Fvs.

1   39.   The method of claim 34, further wherein the candidate binding

2   molecules are Fab fragments.

1   40.   The method of claim 34, further wherein the candidate binding

2   molecules are single V-region domains.

1   41.   The method of claim 33, wherein the candidate binding molecules are
2   scaffolded peptides.

1   42.   The method of claim 33, wherein the candidate binding molecules are
2   mutagenized ligands.

1   43.   The method of claim 34, further wherein the candidate binding
2   molecules are hybrid antibodies that have at least one CDR in a V_H or V_L that is different
3   from the reference antibody and is from a natural antibody repertoire.

1   44.   The method of claim 43, wherein the hybrid antibodies have either a
2   V_H or V_L from the reference antibody and the corresponding V_H or V_L from a natural
3   antibody repertoire.

1   45.   The method of claim 34, further wherein the competitor is a nonhuman
2   antibody and the candidate binding molecules comprise antibodies having at least one human
3   variable region.

1   46.   The method of claim 34, further wherein the competitor is a scaffolded
2   peptide that competes with the reference antibody for binding to the antigen.

1   47.   The method of claim 34, further wherein the competitor is an artificial
2   non-antibody ligand of the antigen that competes with the reference antibody for binding to
3   the antigen.

1   48.   A method of affinity maturation by self-inhibited reactivation to obtain
2   a binding molecule that has a higher affinity for a target binding ensemble member than that
3   of a reference binding molecule, the method comprising:
4   (a) recombinantly altering a population of host cells by
5   (i) introducing into the host cells a competitor binding molecule that
6   binds to a target binding ensemble member with the same specificity as the reference binding
7   molecule,
8   (ii) introducing into the host cells a nucleic acid encoding an auto-
9   inhibited responder complex comprising a responder molecule linked to an inhibitor and to
10 the target binding ensemble member,

11   (iii) introducing into the host cells a library of genes, each encoding a

12   reactivator complex, wherein each gene encodes a reactivator molecule linked to a candidate

13   binding molecule;

14   (b) incubating the host cells under conditions in which the competitor, the

15   auto-inhibited responder-target binding ensemble member complex, and the reactivator

16   library complex are expressed and where the responder molecule is activated when a

17   candidate binding molecule binds to the target binding ensemble member; and

18   (c) detecting cells having a signal from the responder molecule that

19   corresponds to a candidate binding molecule affinity for the target binding ensemble member

20   that is greater than that of the reference binding molecule, thereby identifying a candidate

21   binding molecule with an enhanced affinity for the target binding ensemble member.

1   49.   The method of claim 47, wherein the reference binding molecule is a

2   reference antibody and the target binding ensemble member is an antigen to which the

3   reference antibody specifically binds.

1   50.   The method of claim 49, further wherein the competitor is the

2   reference antibody.

1   51.   The method of claim 49, wherein the reference antibody is an Fab

2   fragment.

1   52.   The method of claim 49, wherein the reference antibody is a single

2   chain Fv (scFv).

1   53.   The method of claim 49, further wherein the candidate binding

2   molecules are single chain Fvs.

1   54.   The method of claim 49, wherein the candidate binding molecules are

2   Fab fragments.

1   55.   The method of claim 49, wherein the candidate binding molecules are

2   single V-region domains.

1   56.   The method of claim 47, wherein the candidate binding molecules are

2   scaffolded peptides.

1   57.   The method of claim 47, wherein the candidate binding molecules are
2   mutagenized natural ligands that specifically bind the target binding ensemble member.

1   58.   The method of claim 49, further wherein the candidate binding
2   molecules are hybrid antibodies that have at least one CDR in a V_H or V_L that is different
3   from the reference antibody and is from a natural antibody repertoire.

1   59.   The method of claim 58, wherein the hybrid antibodies have either a
2   V_H or V_L from the reference antibody and the corresponding V_H or V_L from a natural
3   antibody repertoire.

1   60.   The method of claim 49, further wherein the reference antibody is a
2   nonhuman antibody and the candidate binding molecules are antibodies having at least one
3   human variable region.

1   61.   The method of claim 49, further wherein the competitor is a natural
2   ligand of the target antigen that competes with the reference antibody for binding to the
3   antigen.

1   62.   The method of claim 49, wherein the competitor is a natural ligand of
2   the antigen that competes with the reference antibody for binding to the antigen.